

On the Ontology of Vernal Orange Slime

by Dan Molter

Introduction

This essay is about the ontology of orange slime, found clinging to stumps and vines in early spring. What is it really? I argue here that vernal orange slime (VOS) is a “song”, a kind of biological individual that emerges and evolves when microbes cooperate. First described by Canadian microbiologist Ford Doolittle and American philosopher Austin Booth in 2017, an evolutionary “song” is a symbiotic process that functions as a unit of selection (something upon which natural selection operates), alongside standard Darwinian individuals such as organisms, cells, and mycelia. In microbial consortia, where horizontal gene transfer scrambles parent-offspring lineages and one species can fill in for another in the same functional role, it is “the song not the singers”, which is the target or object of natural selection. I’ll say more in closing about Doolittle’s and Booth’s model of evolutionary individuality and why I think orange slime fits into the “song” category, but I want to begin by saying what vernal orange slime is not.

What VOS is not

First, it is not the same as microbial colonies that grow in sap flows at other times of the year; the succession of microbes that makes VOS a distinct biological phenomenon occurs only in the spring (Weber 2006). The folk name “deer vomit slime” suggests an animal origin and is wholly inaccurate – the deer are not involved, except in case their winter browsing causes a wound from which spring sap flows, sap being the lifeblood of orange slime. Attempts to identify the goo using a field guide are likely to land upon the Latin binomial “*Fusicolla merismoides*”¹ (or in newer publications “*Fusarium merismoides*”). These names are only slightly more accurate. Latin binomials serve as bridges between species in the world and what’s written about them in scientific literature, so their use enables a more precise partitioning of nature than is possible with ordinary language, the latter being inherently vague. While an ontology built from scientific names is more precise, ordinary language ontologies can often be more accurate, for example, when what has been going by a Latin binomial turns out to be a species complex. Dentinger and Luz (2014) showed this to be the case with “*Boletus edulis*”; analyzing the DNA of different mushrooms from a single bag of store-bought porcini, they discovered three new phylogenetic species. Given that no *B. edulis* specimens were in the bag, the common name “porcini” would have been a more accurate label. In the case of VOS, the name “*Fusicolla merismoides*” is neither more precise nor more accurate than “spring flux,” “flux slime”, or “slime volcano”, as *F. merismoides* is probably a species complex; they are not even present in some VOS; and they sing only the latter verses of the orange slime song (see figure 1).

Each patch of VOS is composed of many microbial species, so calling it by any one species name would be analogous to pointing to a wooded hill and uttering the epithet “*Betula pendula*”. Sure, there might be some silver birch in that patch of forest, but it’s certainly not the whole story, and in many forests it’s not even part of the story. So too with vernal orange slime. Calling it by a species name implies that it is a segment of one evolutionary lineage, when it is in fact constituted by the entangled lineages of numerous taxa, the actual species present varying from patch to patch, host to host, and with the advance of the season. The particular name “*Fusicolla merismoides*” is further problematic, because, like “*Boletus edulis*”, it inaccurately denotes multiple species. According to a poster presented at the Fourth International Barcode of Life Conference, “*F. merismoides*... often considered the definitive tree slime fungus, appears to be a large complex of many phylogenetic species. Almost every strain barcoded to date has a different sequence” (Seifert et al 2011).

¹ This paper uses italics to refer to a taxon in nature and quotation marks (without italics) to refer to the taxon name itself. Thus *Fusicolla merismoides* refers to the ascomycete fungus, and “*Fusicolla merismoides*” refers to the Latin binomial.

Roadmap

In the remainder of this article, I will answer questions concerning the biology of vernal orange slime. These answers are based largely on the work of Roland Weber, whose (2006) “On the ecology of fungal consortia of spring sap-flows” is foundational to scientific knowledge on this topic. Professor Weber was kind enough to answer questions for this article. My other sources include the poster mentioned above (Seifert et al 2011), a blog post by Wendy McFadden-Smith “Return of the orange slime” (2011), and a report of nonliving components of VOS by Junhyung Park and colleagues (2017). After summarizing the available science, I will elaborate on Doolittle’s and Booth’s “song not the singer” model of natural selection, and I will argue that vernal orange slime is indeed a song.

How does it arise?

When trees and vines are cut or wounded during fall or winter, they bleed as their sap begins to flow in the spring. This sap, which is up to 1% sugar, flows continuously, providing a reliable source of energy for a succession of microbial communities, similar to the way chemicals spewing from a hydrothermal vent support a vast community of organisms on the ocean floor. Various microbial species arrive in succession, analogous to the way a succession of plant species gradually turn a burned-over patch of ground back into a forest – first come the weeds, then the briars, then fast-growing trees like elm, etc. The first colonizers of spring sap flows are single-celled yeasts, isolated and floating in the sap like plankton in the ocean. Some of these early colonizers are epiphytes already living on the plant’s bark; their populations exploding in the nutrient rich sap. Others are carried in by insects (See image 1), and yet others arrive by way of wind and rain splash. Later in the season, filamentous fungi arrive and biofilms are formed, ending the planktonic phase of the orange slime song. Some of these later colonizers form lattice structures, which provide a niche for yet other yeast species to colonize. This succession of microbes, though highly variable in the species playing the different roles, is faithfully repeated year after year.

What plants host vernal orange slime?

Vernal orange slime has thus far been studied mostly on European Birch and Hornbeam trees (Weber 2006). It is frequently encountered on grape vines (McFadden-Smith 2011, Molter 2012), and one recent study analyzed orange slime growing in the sap flow of a Giant Dogwood (*Cornus controversa*) (Park et al 2017). It likely occurs on numerous other species of woody perennials, but the literature on its occurrence is at present quite sparse.

What species are involved?

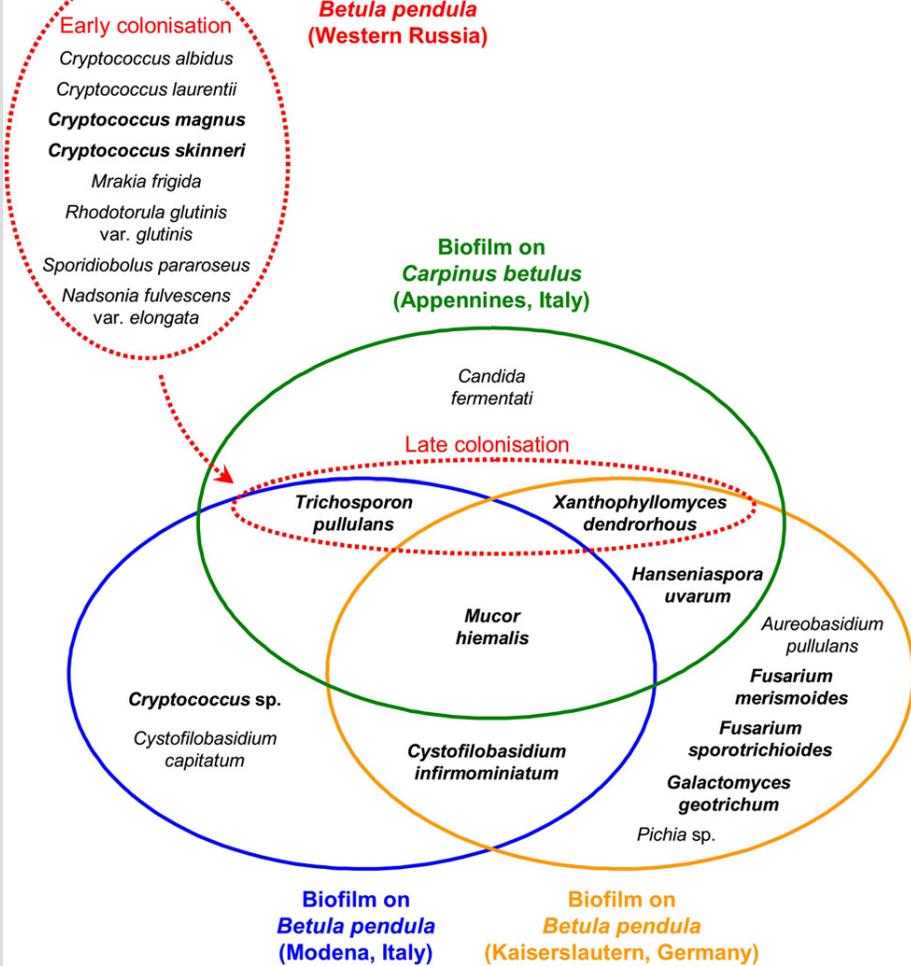
Species reported from VOS include: *Cryptococcus skinneri*, *Cryptococcus magnus*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Cryptococcus macerans*, *Mrakia frigida*, *Rhodotorula glutinis*, *Sporidiobolus pararoseus*, *Nadsonia fulvescens*, *Candida fermentati*, *Trichosporon pullulans*, *Xanthophyllomyces dendrorhous*, *Aureobasidium pullulans*, ‘*Fusicolla merismoides*’, *Fusarium merismoides*, *Fusarium sporotrichioides*, *Fusarium acuminatum*, *Galactomyces geotrichum*, ‘*Rhodomyces dendrorhous*’, ‘*Endomyces vernalis*’, *Cystofilobasidium capitatum*, *Hansenia sporauvarum*, *Cystofilobasidium infirmominiatum*, *Mucor hiemalis*, *Phaffia rhodozyma*, *Epicoccum nigrum*, *Metschnikowia* sp., *Pichai* sp., *Rhizopus* sp., and “other Zygomycetes”. This list is taken from the sources referenced below; the authors speculate that numerous other species are likely involved.

What makes it orange?

The orange coloration, which appears in later stages of development, is due to Astaxanthin and closely related carotenoids, which are produced by yeast species including *Xanthophyllomyces dendrorhous*, the most common species is some late-stage VOS, though not present at all in others. The pigments are likely under selection pressure for their role in relieving oxidative stress.

What gives it structure?

In the early stages of development, the slime is thin and has no structure, though it becomes thickened as the planktonic yeasts proliferate. In later stages, filamentous fungi such as *Mucor hiemalis* and *Fusarium merismoides* expand through the thickened slime, creating a lattice of fibers that provide structural support for other microbes (*Mucor hiemalis* can be seen fruiting in image #2). Park et al (2017) examined VOS under a scanning electron microscope and found extracellular structures made of wax, mucilage, and calcium. Extracellular polymeric substances are a common



The many faces of Vernal Orange Slime

Diagram 1, top left: Geographic variation and chronological succession: fungi from four widely separated instances of VOS. Reused with permission from Weber (2006).

Image 1, top right: In the early planktonic stage of VOS, its component bi-onts are mostly colorless yeasts. These and other bi-onts are often brought in by visiting flies. CC 3.0 Dan Molter

Image 2, below: As filamentous fungi like this *Mucor hiemalis* start growing in the VOS, their hyphae add structure to it. With permission from Roland Weber



Image 3, below: In its later stages we find the fungi with the orange carotenoid pigments that give VOS its name. CC 3.0 Dan Molter



feature of biofilms, making up on average about half the volume of a biofilm, the other half being the microbial cells themselves. Extracellular substances hold microbes together and are under pressure of natural selection (Pedroso 2018). Rooney et al (2020) observed vascular channels distributing nutrients in a biofilm of *E. coli*. Remarkably, the channels reformed after complete mechanical disaggregation of the colony. Structure tends to emerge whenever a system acquires energy from its environment (Horowitz and England 2017). This has led to speculation that life emerges and evolves in complexity wherever in the universe stable energy gradients are found, life being the most efficient way to dissipate energy across a gradient (Margulis and Sagan 2002). Vernal sap flows provide such an energy gradient, but the etiology, physiological role, and selection of structure in VOS remain to be explored.

Is it entirely cooperative?

Interactions between the various parts range from mutualistic to antagonistic. Some species provide structure. Some provide protection from oxidation. But some produce toxins that prevent their close relatives from moving in next door.

A song not the singers

A perennial debate in the foundations of evolutionary theory concerns how the world is divided into individuals. To understand what evolves, we must understand the level of organization at which natural selection operates. Most philosophers of biology now think that natural selection happens at multiple levels and biological individuality therefore occurs at multiple levels (Haber 2012). One of these levels is the holobiont. A Holobiont is a multicellular organism along with all its microbial complement, a category into which everyone reading this article falls. The concept of holobiont was introduced to explain selection on genetic cooperation (Mindell 1992). The genetic machinery needed to carry out a given biological function, such as exploiting a food resource or synthesizing a secondary metabolite, often comes packaged in different species, but so long as the genes are working together, they can encode traits upon which natural selection operates. This was recently shown with two species of fungi from the Berkeley Pit, in Butte MT. The two species cooperate to make a suite of antibiotics that neither can manufacture in isolation (Stierle et al 2017). Doolittle and Booth note that selection on holobionts cannot be driven by traditional Darwinian mechanisms, because those require traits to be transmitted via parent-offspring lineages, especially in the form of genes, while the genes of symbiotic microbes are not passed along this way. In order for natural selection to operate on a holobiont, we need a different means of inheritance. Doolittle and Booth suggest the following:

We propose casting metabolic and developmental interaction patterns, rather than the taxa responsible for them, as units of selection. Such units need not directly reproduce or form parent-offspring lineages: their prior existence has created the conditions under which taxa with the genes necessary to carry out their steps have evolved in large numbers. These taxa or genes will reconstruct the original interaction patterns when favorable conditions occur. Interaction patterns will vary in ways that affect the likelihood of and circumstances under which such reconstruction occurs. Thus, they vary in fitness, and evolution by natural selection will occur at this level (Doolittle and Booth 2017).

According to the 7th principle of holobionts (Bordenstein & Theis 2015), a holobiont must be centered around a single multicellular organism (a macrobe), which anchors the identity of the holobiont as its microbial partners come and go. This precludes overlapping holobionts, which is why I have recently argued that mycorrhizal collectives are not holobionts (Molter 2019). Broader accounts of holobiosis include microbial symbioses (Baedke et al 2020). Regardless of whether it is a holobiont, VOS meets Doolittle's and Booth's description of an evolutionary song: a recurring pattern of metabolic and developmental process upon which natural selection operates. Like a folk song, VOS becomes gradually modified over time. It is not a Darwinian individual, because it does not reproduce (Godfrey Smith 2009), but it nonetheless evolves. Song evolution has recently been modeled (Baptiste & Papale 2021).

Objection 1: VOS is just a symbiotic collective. – Songs differ from symbiotic collectives in the same way a football team differs from a game of football. The game can be played over and over again by different players and different teams; its rules can be changed over time as the game evolves. Strategies get invented and reinvented... The same is true with evolutionary songs. There is indeed a symbiotic collective singing the orange slime song at any given moment, but if we recognize the song as extending through space and time, then we can see that it is not the same symbiotic collective; the singers coming and going as the season advances.

Objection 2: VOS is a holobiont, so we may call it “Fusicolla merismoides” for the same reason we may call human holobionts “Homo sapiens”. – I am grateful to Walt Sturgeon for this objection. While the symbiotic interactions in VOS do count as holobiosis on some uses of the term, there is no organism of any one species that centers the identity of a patch of orange slime. Vernal orange slime seems instead to be like a cascade of overlapping holobionts, only some of which include *F. merismoides* among their parts.

References

- Baedke, J., Fábregas-Tejeda, A., & Nieves Delgado, A. (2020). The holobiont concept before Margulis. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 334(3), 149-155.
- Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol*, 13(8), e1002226.
- Baptiste, E., & Papale, F. (2021). Modeling the evolution of interconnected processes: It is the song and the singers: Tracking units of selection with interaction networks. *BioEssays*, 43(1), 2000077.
- Dentinger, B. T., & Suz, L. M. (2014). What’s for dinner? Undescribed species of porcini in a commercial packet. *PeerJ*, 2, e570.
- Doolittle, W. F., & Booth, A. (2017). It’s the song, not the singer: an exploration of holobiosis and evolutionary theory. *Biology & Philosophy*, 32(1), 5-24.
- Godfrey-Smith, P. (2009). *Darwinian populations and natural selection*. Oxford University Press.
- H. Haber, M. (2012). Multilevel lineages and multidimensional trees: the levels of lineage and phylogeny reconstruction. *Philosophy of Science*, 79(5), 609-623.
- Horowitz, J. M., & England, J. L. (2017). Spontaneous fine-tuning to environment in many-species chemical reaction networks. *Proceedings of the National Academy of Sciences*, 114(29), 7565-7570.
- Margulis, L., & Sagan, D. (2002). *Acquiring genomes: A theory of the origin of species*. Basic books.
- McFadden-Smith, Wendy. (2011) “Return of the orange slime”. <https://blogs.cornell.edu/nygrapeupdate/2014/05/23/return-of-the-orange-slime/>
- Mindell D.P. (1992) Phylogenetic consequences of symbioses: eukarya and eubacteria are not monophyletic taxa. *Biosystems* 27(1):53–62
- Molter, D. J. (2019). On mycorrhizal individuality. *Biology & Philosophy*, 34(5), 1-16.
- Molter, D. (2012) Observation #93656.mushroomobserver.org. <https://mushroomobserver.org/93656>
- Park, J., Kwon, J. H., Kim, H. R., Kwon, O., Koh, S. H., Kim, P. G., ... & Kim, K. W. (2017). Fungal proliferation and calcium accumulation in the orange slime of *Cornus controversa*. *Forest science and technology*, 13(4), 192-199.
- Pedroso, M. (2018). Forming lineages by sticking together. *Philosophy, Theory, and Practice in Biology*, 11(016).
- Rooney, L. M., Amos, W. B., Hoskisson, P. A., & McConnell, G. (2020). Intra-colony channels in *E. coli* function as a nutrient uptake system. *The ISME journal*, 14(10), 2461-2473.
- Keith Seifert, Tom Gräfenhan, Kathie Hodge, Brett Summere, Yosuke Degawa, and Wendy McFadden-Smith (2011). *The International Tree Slime Project DNA barcoding of fungal volcanoes*. <http://cesanluisobispo.ucanr.edu/files/300145.pdf>
- Stierle, A. A., Stierle, D. B., Decato, D., Priestley, N. D., Alverson, J. B., Hoody, J. ... & Klepacki, D. (2017). The berkeleylactones, antibiotic macrolides from fungal coculture. *Journal of natural products*, 80(4), 1150-1160.
- Weber, R. W. (2006). On the ecology of fungal consortia of spring sap-flows. *Mycologist*, 20(4), 140-143.